REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

The September 24, 2002, and October 2, 2002, telephonic interviews between Examiner Sisson, applicants, and applicants' undersigned attorney, with and without applicants, respectively, are gratefully acknowledged. The substance of these interviews is summarized below.

Single-nucleotide polymorphisms (SNPs) are the most frequent type of variation in the human genome with an estimated frequency of one to two polymorphic nucleotides per kilobase. SNPs can serve as genetic markers for identifying disease genes by linkage studies in families, linkage disequilibrium in isolated populations, association analysis of patients and controls, and loss-of-heterozygosity studies in tumors. Although some SNPs in single genes are associated with heritable diseases such as cystic fibrosis, sickle cell anemia, colorectal cancer, and retinitis pigmentosa, most SNPs are "silent". They can alter phenotype by either controlling the splicing together of exon from intron-containing genes or changing the way mRNA folds. Recently, there has been increasing knowledge of the genetic basis of SNPs for individual differences in drug response. Insights into differences between alleles or mutations present in different individuals can also illuminate the interplay of environment with disease susceptibility. For example, in the p*53* tumor suppressor gene, over 400 mutations have been found to be associated with tumors and used to determine individuals with increased cancer risk. All these applications involve the analysis of a large number of samples and will eventually require rapid, inexpensive, and highly automated methods for genotyping analysis.

Because of the importance of identifying SNPs, a number of gel-based methods have been described for their detection and genotyping. These methods include single strand conformational polymorphism analysis, heteroduplex analysis, denaturing gradient gel electrophoresis, and chemical or enzyme mismatch modification assays. To facilitate large-scale SNP identification, new technologies are being developed to replace the conventional gel-based re-sequencing methods. Perhaps the most widely employed techniques currently used for SNP identification are array hybridization assays, such as allele specific oligonucleotide microarrays in miniaturized assays. This approach relies on the capacity to distinguish a perfect match from a single base mismatch by hybridization of target

DNA to a related set of four groups of oligonucleotides that are identical except for the base centrally located in the oligonucleotide. Mismatches in the central base of the oligonucleotide sequence have a greater destabilizing effect than mispairing at distal positions during hybridization. Thus, this strategy developed by Affymetrix utilizes a set of four oligonucleotides for each base to re-sequence. For example, a 10-kb gene requires a microarray of 40,000 oligos that can be accomplished by on-chip photolithographic synthesis. The mutation detection is based on the development of a two-color labeling scheme, in which the reference DNA is labeled with phycoerythrin (red) during the PCR amplification, while the target DNA is labeled with fluorescein (green). Both reference and target samples can then be hybridized in parallel to separate chips with identically synthesized arrays or co-hybridized to the same chip. The signal of hybridization of fluorescent products is recorded through confocal microscopy. Comparison of the images for a target sample and reference sample can yield the genotype of the target sample for thousands of SNPs being tested. By processing co-hybridization of the reference and target samples together, experimental variability during the subsequent fragmentation, hybridization, washing, and detection steps can be minimized to make array hybridization more reproducible. The interpretation of the result is based on the ratios between the hybridization signals from the reference and the target DNA with each probe.

Despite the impressive technology that is emerging for the hybridization to oligonucleotide arrays, potential problems with these approaches exist due to several factors. One limiting factor originates from the inherent properties of the nucleic acid hybridization. The efficiency of hybridization and thermal stability of hybrids formed between the target DNA and a short oligonucleotide probe depend strongly on the nucleotide sequence of the probe and the stringency of the reaction conditions. Furthermore, the degree of destabilization of the hybrid molecule by a mismatched base at one position is dependent on the flanking nucleotide sequence. As a result, it would be difficult to design a single set of hybridization conditions that would provide optimal signal intensities and discrimination of a large number of sequence variants simultaneously. This is particularly true for human genomic DNA which is present either in heterozygous or homozygous form. In addition, the necessity of using DNA chips composed of tens of oligonucleotide probes per analyzed nucleotide position has led to a complex setup of assays and requires mathematical algorithms for interpretation of the data.

Another popular method for high-throughput SNP analysis is called 5' exonuclease assay in which two fluorogenic probes, double-labeled with a fluorescent

reporter dye (FAM or TET) and a quencher dye (TAMRA) are included in a typical PCR amplification. During PCR, the allele-specific probes are cleaved by the 5' exonuclease activity of *Taq* DNA polymerase, only if they are perfectly annealed to the segment being amplified. Cleavage of the probes generates an increase in the fluorescence intensity of the reporter dye. As a result, both report fluorescence that can be plotted and segregated to determine the template genotype. The advantage of this approach is to virtually eliminate post-PCR processing. However, the apparent drawbacks of this technique relate to the time and expense of establishing and optimizing conditions for each locus.

Another widely accepted method to identify SNPs is called single nucleotide primer extension (SNuPE), also known as minisequencing. This technique involves the hybridization of a primer immediately adjacent to the polymorphic locus, extension by a single dideoxynucleotide, and identification of the extended primer. An advantage of this approach, compared to hybridization with oligonucleotide probes, is that all variable nucleotides are identified with optimal discrimination using the same reaction conditions. Consequently, at least one order of magnitude of higher power for discriminating between genotyping is available using this method than with hybridization of allele-specific oligonucleotide probes in the same array format.

Since the first introduction of SNuPE for the identification of genetic disease, several new detection methods have been developed including luminous detection, colorimetric ELISA, gel-based fluorescent assays, homogeneous fluorescent detection, flow cytometry-based assays and HPLC analysis. Recently, a combination of single nucleotide primer extension and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOFMS) detection has been developed. This approach allows the determination of SNP sequences by measuring the mass difference between the known primer mass and the extended primer mass using MALDI-TOFMS. Discrimination of mass differences of less than 1 part in 1,000 is required to determine which of the four dideoxynucleotide triphosphate bases (ddNTPs), dideoxy-cytidine triphosphate (ddCTP), dideoxy-thymidine triphosphate (ddTTP), dideoxy-adenosine triphosphate (ddATP), and dideoxy-guanosine triphosphate (ddGTP) reacted to extend the primer. A desired capability of this technique includes the analysis of heterozygotes where two different bases are present at the same nucleotide position. The MALDI-TOFMS measurement requires the discrimination of two mass-resolved species that represent the addition of both bases complementary to those at the SNP site. This requires MALDI-TOFMS methods incorporating high mass resolution capabilities and enhanced sensitivity. Compared to the

detection of a fluorescence-labeled nucleotide by non-mass spectrometric methods, mass detection is faster, and less laborious without the need for modified or labeled bases. Mass detection offers advantages in accuracy, specificity, and sensitivity. Recently, a chip-based primer extension combined with mass spectrometry detection for genotyping was performed on a 1-μL scale in the wells contained within a microchip without using conventional sample tubes and microtiter plates. This miniaturized method clearly provides another potential for high-throughput and low cost identification of genetic variations.

Current methods exist for the identification of SNPs using electrospray for the mass detection of the extended primers. These methods are similar to MALDI-TOFMS in that mass measurements to within 1 part in 1,000 are required to discriminate which base extended the oligonucleotide primer. Also, electrospray ionization of large oligonucleotides is difficult, requiring someone highly skilled in the interpretation of the data.

As SNPs are used in applications such as gene location, drug resistance testing, disease diagnosis, and identity testing, a concomitant increase in the rate of routine SNP characterization will be necessary. Pooling of DNA from ten to thousands of individuals into one sample before genotyping is a valuable means of streamlining large-scale SNP genotyping in disease association studies. The results from pooling are interpreted as a representation of the allele frequency distribution in the individual samples and can be used to validate a candidate SNP as common or rare or merely detect the presence of a particular variation in the pooled DNA sample. Quantitation of small molecules by electrospray ionization is well known to provide high sensitivity and linear responses over 3-4 orders of magnitude. The electrospray ionization/mass spectrometry procedure, in accordance with the present invention, can be used to accurately quantify small molecules for SNP genotyping and can provide an advantage when analyzing pooled DNA samples for the purpose of determining SNP allele frequencies.

The present invention is a single base DNA variation detection method which overcomes the above-noted deficiencies in prior techniques.

The rejection of claims 1-20 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed.

During the first telephonic interview with regard to this rejection, concern was raised about the lack of clarity in the claims with respect to whether the method involves measuring nucleotides that have not been incorporated during the primer extension reaction. Claim 1 has now been amended to clarify that the method involves "measuring" the amounts of each type of the "unreacted" plurality of types of nucleotide analogs remaining in the

extension solution after said extending, comparing the amounts of each type of the "unreacted" plurality of types of nucleotide analogs remaining in the extension solution after said extending to the amounts of each type of the plurality of types of nucleotide analogs in a control sample which did not undergo said step of extending, and identifying the type of "unreacted" plurality of types of nucleotide analogs which is present in the extension solution after said extending in an amount less than in the control sample as the nucleotide added to the oligonucleotide primer at the active site so that the nucleotide at the active site of the target nucleic acid molecule is determined. Applicants submit that, as amended above, the claims of the present application overcome all bases for rejection.

During the second telephonic interview, U.S. Patent No. 4,863,849 to Melamede ("Melamede") was cited of interest. Melamede describes an automatable method for sequencing DNA or RNA that does not require radioactivity or gel electrophoresis. Melamede, however, does not preclude patent protection for the subject matter of the present application. In particular, Melamede does not in any way suggest a method of detecting single nucleotide polymorphisms using "a plurality of types of nucleotide analogs", as claimed by applicants. In addition, Melamede does not teach a number of features found in applicants' dependent claims, including use of dideoxynucleotide analogs (claim 2), electrospray (claim 3), specific electrospray structure (claims 4-12), a metal chelating resin (claim 14-17), or mass spectrometry (claims 54-55). Accordingly, Melamede provides no basis for rejecting the pending claims.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Jan. 2, 2003 Date	Ruth R. Smith

Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made herein to claims 1, 3, 12, 18, and 20, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

Please amend claims 1, 3, 12, 18, and 20 as follows:

1. (Amended) A method of detecting single nucleotide polymorphisms comprising:

providing a target nucleic acid molecule;

providing an oligonucleotide primer complementary to a portion of the target nucleic acid molecule;

providing a nucleic acid polymerizing enzyme;

providing a plurality of types of nucleotide analogs;

blending the target nucleic acid molecule, the oligonucleotide primer, the nucleic acid polymerizing enzyme, and the plurality of types of nucleotide analogs[, each type being present in a first amount,] to form an extension solution where the oligonucleotide primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add the plurality of types of nucleotide analogs to the primed target nucleic acid molecule at an active site;

extending the oligonucleotide primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide primer at the active site to form an extended oligonucleotide primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site;

[determining] measuring the amounts of each type of the unreacted plurality of types of nucleotide analogs remaining in the extension solution after said extending[, each type being a second amount];

comparing the [first and second] amounts of each type of the unreacted plurality of types of nucleotide [analog] analogs remaining in the extension solution after said

extending to the amounts of each type of the plurality of types of nucleotide analogs in a control sample which did not undergo said step of extending; and

identifying the type of unreacted plurality of types of nucleotide [analog where the first and second amounts differ] analogs which is present in the extension solution after said extending in an amount less than in the control sample as the nucleotide added to the oligonucleotide primer at the active site so that the nucleotide at the active site of the target nucleic acid molecule is determined.

3. (Amended) A method according to claim 1, wherein said [determining] measuring is carried out by electrospraying the extension solution.

12. (Amended) A method according to claim 3, wherein said [determining] measuring comprises detecting the amounts of each type of the unreacted plurality of types of nucleotide analogs in the electrospray.

18. (Amended) A method according to claim 3 further comprising:

evaporating [water from] the extension solution[, leaving] to leave a [residue] residual material and [sonicating] reconstituting the [residue] residual material in water after said extending and before the electrospraying.

20. (Amended) A method according to claim 1 [further comprising:

amplifying] , wherein said providing a target nucleic acid molecule comprises:

providing the target nucleic acid molecule [by] in a sample and

subjecting the sample to a polymerase chain reaction [prior to said blending] to amplify the nucleic acid molecule.